CLAIMS

1. A method for making a polynucleotide (X1X2) comprising two nucleotide segments of interest, X1 and X2, wherein X2 in X1X2 is immediately 3' to X1, from a nucleic acid molecule including X1 and the same or a different nucleic acid molecule including X2, wherein if X1 and X2 originate on the same molecule, they are not contiguous, the method comprising:

(a) amplifying a first double stranded nucleic acid segment X1, which segment comprises a sense and an antisense nucleic acid strand, with a first primer set, which primer set comprises (i) a forward primer, PFX1, which hybridizes to the 3' end of the antisense strand of X1 and (ii) a reverse primer, PRX1, which hybridizes to the 3' end of the sense strand of X1;

(b) amplifying a second double stranded nucleic acid segment, X2, which segment comprises a sense and an antisense nucleic acid strand, with a second primer set, which second primer set comprises (i) a forward primer, PFX2, which hybridizes to the 3' end of the antisense strand of X2 and (ii) a reverse primer, PRX2, which hybridizes to the 3' end of the sense strand of X2;

(c) isolating the X1 and X2 products of steps (a) and (b)

(d) performing PCR in a single reaction vessel, said vessel comprising the isolated X1 and X2 products of step (c) in stoichiometric amounts and primers PFX1, PRX2, and a fusion primer, which fusion primer has the nucleotide sequence of PRX1 preceded at its 5' end by the sequence of the complement of PFX2, said PFX2 complement termed PFX2', wherein PCR performed in this single vessel results in amplification of an intermediate double stranded polynucleotide X1UR, which intermediate comprises a sense and an antisense nucleic acid strand, and which comprises the double stranded nucleic acid segment X1 and a 5' double stranded nucleic acid segment of X2, wherein the 3' end of X1 is fused to the 5' segment of X2, and wherein said reaction also results in amplification of the X1UR intermediate to make X1X2 by denaturing and annealing X1UR and X2 to form annealed species and then extending and amplifying said annealed species using DNA polymerase

possessing both 5'-3' polymerase activity and 5'-3' exonuclease activity and primers PFX1 and PRX2.

2. A method for making a polynucleotide (X1X2) comprising two nucleotide segments of interest, X1 and X2, wherein X2 in X1X2 is immediately 3' to X1, from a nucleic acid molecule including X1 and the same or a different nucleic acid molecule including X2, wherein if X1 and X2 originate on the same molecule, they are not contiguous, the method comprising:

(a) amplifying a first double stranded nucleic acid segment X1, which segment comprises a sense and an antisense nucleic acid strand, with a first primer set, which primer set comprises (i) a forward primer, PFX1, which hybridizes to the 3' end of the antisense strand of X1 and (ii) a reverse primer, PRX1, which hybridizes to the 3' end of the sense strand of X1;

(b) amplifying a second double stranded nucleic acid segment, X2, which segment comprises a sense and an antisense nucleic acid strand, with a second primer set, which second primer set comprises (i) a forward primer, PFX2, which hybridizes to the 3' end of the antisense strand of X2 and (ii) a reverse primer, PRX2, which hybridizes to the 3' end of the sense strand of X2;

(c) isolating the X1 and X2 products of steps (a) and (b)

(d) performing PCR in a single reaction vessel, said vessel comprising the isolated X1 and X2 products of step (c) in stoichiometric amounts and primers PRX1, PFX2, and a fusion primer, which fusion primer has the nucleotide sequence of PFX2 preceded at its 5' end by the sequence of the complement of PRX1, said PRX1 complement termed PRX1', wherein PCR performed in this single vessel results in amplification of an intermediate double stranded polynucleotide DRX2, which intermediate comprises a sense and an antisense nucleic acid strand, and which comprises the double stranded nucleic acid segment X2 and a 3' double stranded nucleic acid segment of X1, wherein the 5' end of X2 is fused to the 3' segment of X1, and said reaction also results in amplification of the DRX2 intermediate to make X1X2 by denaturing and annealing DRX2 and X1 to form annealed species and then

extending and amplifying said annealed species using DNA polymerase possessing both 5'-3' polymerase activity and 5'-3' exonuclease activity and primers PRX1 and PFX2.

3. A method for making a polynucleotide (X1X2) comprising two nucleotide segments of interest, X1 and X2, wherein X2 in X1X2 is immediately 3' to X1, from a nucleic acid molecule including X1 and the same or a different nucleic acid molecule including X2, wherein if X1 and X2 originate on the same molecule, they are not contiguous, the method comprising:

(a) amplifying a first double stranded nucleic acid segment X1, which segment comprises a sense and an antisense nucleic acid strand, with a first primer set, which primer set comprises (i) a forward primer, PFX1, which hybridizes to the 3' end of the antisense strand of X1 and (ii) a reverse primer, PRX1, which hybridizes to the 3' end of the sense strand of X1;

(b) amplifying a second double stranded nucleic acid segment, X2, which segment comprises a sense and an antisense nucleic acid strand, with a second primer set, which second primer set comprises (i) a forward primer, PFX2, which hybridizes to the 3' end of the antisense strand of X2 and (ii) a reverse primer, PRX2, which hybridizes to the 3' end of the sense strand of X2;

(c) amplifying an intermediate double stranded polynucleotide X1UR, which intermediate comprises a sense and an antisense nucleic acid strand, and which comprises the double stranded nucleic acid segment X1 and a 5' double stranded nucleic acid segment of X2, wherein the 3' end of X1 is fused to the 5' segment of X2, with a third primer set, which third primer set comprises (i) PFX1 and (ii) a fusion primer, which fusion primer has the nucleotide sequence of PRX1 preceded at its 5' end by the sequence of the complement of PFX2, said PFX2 complement termed PFX2'; and

(d) making and amplifying X1X2 by denaturing and annealing X1UR and X2 to form annealed species and then extending and amplifying said annealed species using DNA polymerase and primers PFX1 and PRX2.

4. A method for making a polynucleotide (X1X2) comprising two nucleotide segments of interest, X1 and X2, wherein X2 in X1X2 is immediately 3' to X1, from a nucleic acid molecule including X1 and the same or a different nucleic acid molecule including X2, wherein if X1 and X2 originate on the same molecule, they are not contiguous, the method comprising:

(a) amplifying a first double stranded nucleic acid segment X1, which segment comprises a sense and an antisense nucleic acid strand, with a first primer set, which primer set comprises (i) a forward primer, PFX1, which hybridizes to the 3' end of the antisense strand of X1 and (ii) a reverse primer, PRX1, which hybridizes to the 3' end of the sense strand of X1;

(b) amplifying a second double stranded nucleic acid segment, X2, which segment comprises a sense and an antisense nucleic acid strand, with a second primer set, which second primer set comprises (i) a forward primer, PFX2, which hybridizes to the 3' end of the antisense strand of X2 and (ii) a reverse primer, PRX2, which hybridizes to the 3' end of the sense strand of X2;

(c) amplifying an intermediate double stranded polynucleotide DRX2, which intermediate comprises a sense and an antisense nucleic acid strand, and which comprises the double stranded nucleic acid segment X2 and a 3' double stranded nucleic acid segment of X1, wherein the 5' end of X2 is fused to the 3' segment of X1, with a third primer set, which third primer set comprises (i) PRX2 and (ii) a fusion primer, which fusion primer has the nucleotide sequence of PFX2 preceded at its 5' end by the sequence of the complement of PRX1, said PRX1 complement termed PRX1'; and

(d) making and amplifying X1X2 by denaturing and annealing DRX2 and X2 to form annealed species and then extending and amplifying said annealed species using DNA polymerase and primers PRX1 and PFX2.

5. A method for making a polynucleotide (X1X2) comprising two nucleotide segments of interest, X1 and X2, wherein X2 in X1X2 is immediately 3' to X1, from a nucleic acid molecule including X1 and the same or a different nucleic acid

molecule including X2, wherein if X1 and X2 originate on the same molecule, they are not contiguous, the method comprising;

(a) amplifying a first double stranded nucleic acid segment, X2, which segment comprises a sense and an antisense nucleic acid strand, with a second primer set, which second primer set comprises (i) a forward primer, PFX2, which hybridizes to the 3' end of the antisense strand of X2 and (ii) a reverse primer, PRX2, which hybridizes to the 3' end of the sense strand of X2;

(b) amplifying an intermediate double stranded polynucleotide X1UR, which intermediate comprises a sense and an antisense nucleic acid strand, and which comprises the double stranded nucleic acid segment X1 and a 5' double stranded nucleic acid segment of X2, wherein the 3' end of X1 is fused to the 5' segment of X2, with a third primer set, which third primer set comprises (i) a forward primer, PFX1, which hybridizes to the 3' end of the antisense strand of X1 and (ii) a fusion primer, which fusion primer has the nucleotide sequence of a reverse primer, PRX1, which hybridizes to the 3' end of the sense strand of X1, preceded at its 5' end by the sequence of the complement of PFX2, said PFX2 complement termed PFX2'; and

(c) making and amplifying X1X2 by denaturing and annealing X1UR and X2 to form annealed species and then extending and amplifying said annealed species using DNA polymerase and primers PFX1 and PRX2.

6. A method for making a polynucleotide (X1X2) comprising two nucleotide segments of interest, X1 and X2, wherein X2 in X1X2 is immediately 3' to X1, from a nucleic acid molecule including X1 and the same or a different nucleic acid molecule including X2, wherein if X1 and X2 originate on the same molecule, they are not contiguous, the method comprising:

(a) amplifying a first double stranded nucleic acid segment X1, which segment comprises a sense and an antisense nucleic acid strand, with a first primer set, which primer set comprises (i) a forward primer, PFX1, which hybridizes to the 3' end of the antisense strand of X1 and (ii) a reverse primer, PRX1, which hybridizes to the 3' end of the sense strand of X1;

(b) amplifying an intermediate double stranded polynucleotide DRX2, which intermediate comprises a sense and an antisense nucleic acid strand, and which comprises the double stranded nucleic acid segment X2 and a 3' double stranded nucleic acid segment of X1, wherein the 5' end of X2 is fused to the 3' segment of X1, with a third primer set, which third primer set comprises (i) a reverse primer, PRX2, which hybridizes to the 3' end of the sense strand of X2 and (ii) a fusion primer, which fusion primer has the nucleotide sequence of a forward primer, PFX2, which hybridizes to the 3' end of the antisense strand of X2 preceded at its 5' end by the sequence of the complement of PRX1, said PRX1 complement termed PRX1'; and

(c) making and amplifying X1X2 by denaturing and annealing DRX2 and X2 to form annealed species and then extending and amplifying said annealed species using DNA polymerase and primers PRX1 and PFX2.

7. The method of any of claims 1-6 characterized in that the segments X1 and X2 are both included in the same nucleic acid molecule.

8. The method of claim 7 characterized in that said nucleic acid molecule is the genome of a mammalian, preferably a human being.

9. The method of any of claims 1-8 characterized in that said polynucleotide (X1X2) encodes a biologically active polypeptide.

10. The method of claim 9 characterized in that said polynucleotide (X1X2) encodes a polypeptide having the activity of chorionic gonadotropin (CG); luteinizing hormone (LH), follicle stimulating hormone (FSH) or a polypeptide having the activity of both LH and FSH; thyroid stimulating hormone (TSH); alfa-interferon (INF-α), beta-interferon (INF-β) or a polypeptide having the activity of both INF-α and INF-β.

11. The method of any of claims 1-8 characterized in that the segment X1 encodes a signal sequence and X2 encodes a biologically active polypeptide.

12. A method for manufacturing a recombinant protein encoded by the polynucleotide (X1X2) which comprises inserting in a suitable expression vector the polynucleotide (X1X2) obtained by the method according to any of claims 1-6.

13. A nucleic acid molecule comprising the polynucleotide sequence set forth in SEQ ID NO: 20.

14. A vector comprising the nucleic acid molecule of claim 13.

15. A mammalian host cell transformed to express the polynucleotide of SEQ ID NO: 20.

16. A polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 27.

17. An oligonucleotide having the sequence set forth in SEQ ID NO: 11.

18. An oligonucleotide having the sequence set forth in SEQ ID NO: 12.

19. An oligonucleotide having the sequence set forth in SEQ ID NO: 30 or SEQ ID NO: 31.

20. A polypeptide encoded by one of the oligonucleotides according to claim 19.

21. A polypeptide having the activity of the polypeptide according to claim 20.

22. A method for making a polynucleotide (X1X2) comprising two nucleotide segments of interest, X1 and X2, wherein X2 in X1X2 is immediately 3' to X1, from a nucleic acid molecule including X1 and the same or a different nucleic acid molecule including X2, wherein if X1 and X2 originate on the same molecule, they are not contiguous, the method comprising;

(a) amplifying at least one intermediate double stranded polynucleotide selected from the group consisting of X1UR and DRX2, wherein UR is a 5' segment of X2 having at least 12 nucleotides and DR is a 3' segment of X1 having at least 12 nucleotides, in a polymerase chain reaction using the following primer sets:

(i) for X1UR; a forward primer, PFX1, which hybridizes to the 3' end of the antisense strand of X1 and a fusion primer, which fusion primer has the nucleotide sequence of a reverse primer, PRX1, which hybridizes to the 3' end of

the sense strand of X1, preceded at its 5' end by the sequence of the complement of PFX2, said PFX2 complement termed PFX2'; and

(ii) for DRX2, a reverse primer, PRX2, which hybridizes to the 3' end of the sense strand of X2 and a fusion primer, which fusion primer has the nucleotide sequence of a forward primer, PFX2, which hybridizes to the 3' end of the antisense strand of X2 preceded at its 5' end by the sequence of the complement of PRX1, said PRX1 complement termed PRX1';

(b) forming Species A selected from the group of;

(i) the sense strand of X1UR annealed to the antisense strand of X2 where the intermediate is X1UR;

(ii) the sense strand of X1 annealed to the antisense strand of DRX2 where the intermediate is DRX2; and

(iii) the sense strand of X1UR annealed to the antisense strand of DRX2 where the intermediate is X1UR and DRX2;

(c) forming Species B selected from the group of:

(i) the antisense strand of X1UR annealed to the sense strand of X2 where the intermediate is X1UR;

(ii) the antisense strand of X1 annealed to the sense strand of DRX2 where the intermediate is DRX2; and

(iii) the antisense strand of X1UR annealed to the sense strand of DRX2 where the intermediate is X1UR and DRX2; and

(d) by a polymerase chain reaction performed under predetermined polymerization conditions, extending Species A using a polymerase enzyme possessing 5'-3' polymerase activity to form X1X2, and extending Species B using primers PFX1 and PRX2 and a polymerase enzyme possessing both 5'-3' polymerase activity and 5'-3' exonuclease activity to form X1X2.

23. A method for making a polynucleotide (X1X2) comprising two nucleotide segments of interest, X1 and X2, wherein X2 in X1X2 is immediately 3' to X1, from a nucleic acid molecule including X1 and the same or a different nucleic acid

molecule including X2, wherein if X1 and X2 originate on the same molecule, they are not contiguous, the method comprising;

(a) amplifying at least one intermediate double stranded polynucleotide selected from the group consisting of X1UR and DRX2, wherein UR is a 5' segment of X2 having at least 12 nucleotides and DR is a 3' segment of X1 having at least 12 nucleotides, in a polymerase chain reaction using the following primer sets:

(i) for X1UR; a forward primer, PFX1, which hybridizes to the 3' end of the antisense strand of X1 and a fusion primer, which fusion primer has the nucleotide sequence of a reverse primer, PRX1, which hybridizes to the 3' end of the sense strand of X1, preceded at its 5' end by the sequence of the complement of PFX2, said PFX2 complement termed PFX2'; and

(ii) for DRX2, a reverse primer, PRX2, which hybridizes to the 3' end of the sense strand of X2 and a fusion primer, which fusion primer has the nucleotide sequence of a forward primer, PFX2, which hybridizes to the 3' end of the antisense strand of X2 preceded at its 5' end by the sequence of the complement of PRX1, said PRX1 complement termed PRX1';

(b) annealing at least one of the following;

(i) the sense strand of X1UR and the antisense strand of X2 to form Species A;

(ii) the sense strand of X1 and the antisense strand of DRX2 to form Species A; and

(iii) the sense strand of X1UR and the antisense strand of DRX2 to form Species A;

(iv) the antisense strand of X1UR and the sense strand of X2 to form Species B;

(v) the antisense strand of X1 and the sense strand of DRX2 to form Species B; and

(iii) the antisense strand of X1UR and the sense strand of DRX2 to form Species A; and

(c) by a polymerase chain reaction performed under predetermined polymerization conditions, extending Species A using a polymerase enzyme possessing 5'-3' polymerase activity to form X1X2, and extending Species B using primers PFX1 and PRX2 and a polymerase enzyme possessing both 5'-3' polymerase activity and 5'-3' exonuclease activity to form X1X2.

24. The method of claim 22 or 23 characterized in that the segments X1 and X2 are both included in the same nucleic acid molecule.

25. The method of claim 24 characterized in that said nucleic acid molecule is the genome of a mammalian, preferably a human being.

26. The method of any of claims 22-25 characterized in that said polynucleotide (X1X2) encodes a biologically active polypeptide.

27. The method of claim 26 characterized in that said polynucleotide (X1X2) encodes a polypeptide having the activity of chorionic gonadotropin (CG), luteinizing hormone (LH), follicle stimulating hormone (FSH) or thyroid stimulating hormone (TSH).

28. The method of any of claims 22-27 characterized in that the segment X1 encodes a signal sequence and X2 encodes a biologically active polypeptide.

29. A method for manufacturing a recombinant protein encoded by the polynucleotide (X1X2) which comprises inserting in a suitable expression vector the polynucleotide (X1X2) obtained by the method according to any of claims 22-27.